

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED		1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY DTIC SELECTED		3. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release Distribution Unlimited	
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE 4 1995		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) F		7a. NAME OF MONITORING ORGANIZATION	
6a. NAME OF PERFORMING ORGANIZATION DIVISION OF BIOCHEMISTRY		7b. ADDRESS (City, State, and ZIP Code)	
6b. OFFICE SYMBOL (if applicable) SGRD-UWG		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
6c. ADDRESS (City, State, and ZIP Code) WALTER REED ARMY INSTITUTE OF RESEARCH WASHINGTON, DC 20307-5100		10. SOURCE OF FUNDING NUMBERS	
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND		PROGRAM ELEMENT NO.	
8b. OFFICE SYMBOL (if applicable)		PROJECT NO.	
8c. ADDRESS (City, State, and ZIP Code) FORT DETRICK, FREDERICK, MD 21701 WALTER REED ARMY INSTITUTE OF RESEARCH WASHINGTON, DC 20307-5100		TASK NO.	
11. TITLE (Include Security Classification) Vaccination against cholesterol: Immunologic modulation of diet-induced hypercholesterolemia and atherosclerosis		WORK UNIT ACCESSION NO.	
12. PERSONAL AUTHOR(S) Alving, et al		14. DATE OF REPORT (Year, Month, Day)	
13a. TYPE OF REPORT		15. PAGE COUNT	
13b. TIME COVERED FROM _____ TO _____			
16. SUPPLEMENTARY NOTATION			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	cholesterol, immunologic,
19. ABSTRACT (Continue on reverse if necessary and identify by block number)			
<p>Summary: Immunization of rabbits with a protein-free formulation consisting of liposomes containing 71% cholesterol and lipid A as an adjuvant induced antibodies that recognized highly purified nonoxidized crystalline cholesterol and rabbit VLDL/LDL by ELISA. In rabbits that were fed an atherogenic diet containing 0.5%-1.0% cholesterol, a marked diminution of hypercholesterolemia (as much as 979 mg/dl lower) was observed in the immunized animals when compared to nonimmunized controls. Analysis of aortic fatty streaks by automated morphometric probability-of-occurrence mapping of sudanophilia showed significantly diminished lesions in vaccinees in most areas of the aorta.</p>			
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL		22b. TELEPHONE (Include Area Code)	
		22c. OFFICE SYMBOL	

Vaccination against cholesterol: immunologic modulation of diet-induced hypercholesterolemia and atherosclerosis in rabbits

Carl R. Alving¹, Glenn M. Swartz Jr.¹, Nabila M. Wassef¹, Edward E. Herderick³, Renu Virmani², Frank D. Kolodgie², Gary R. Matyas¹, Jorge L. Ribas², Julie R. Kenner¹ and J. Frederick Cornhill^{3,4}

¹Department of Membrane Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20307-5100;

²Armed Forces Institute of Pathology, Washington, DC; ³Ohio State University, Columbus, Ohio; and ⁴Cleveland Clinic Foundation, Cleveland, Ohio, USA

Abstract. Immunization of rabbits with a protein-free formulation consisting of liposomes containing 71% cholesterol and lipid A as an adjuvant induced antibodies that recognized highly purified nonoxidized crystalline cholesterol and rabbit VLDL/IDL by ELISA. In rabbits that were fed an atherogenic diet containing 0.5–1.0% cholesterol, a markedly lower hypercholesterolemia (as much as 979 mg/dl less) was observed in the immunized animals than in nonimmunized controls. Analysis of aortic fatty streaks by automated morphometric probability-of-occurrence mapping of sudanophilia showed significantly smaller lesions in vaccinees in most areas of the aorta.

Background

Cholesterol was first proposed in 1925 as an antigenic molecule against which specific antibodies could be induced in experimental animals in the presence of heterologous proteins that served as carriers [1]. The observation of apparent antibodies to cholesterol generated a considerable early interest in lipid immunology, and some controversy, which culminated more recently in the development of specific immunoassays for a wide range of steroid hormones (reviewed in [2]). In 1988, monoclonal antibodies to cholesterol were developed by immunization of mice with protein-free liposomes heavily loaded (71 mol%) with unconjugated highly purified nonoxidized cholesterol as an antigen and lipid A, the endotoxic portion of bacterial lipopolysaccharide, as an adjuvant [3]. The antibodies recognized purified, nonoxidized, crystalline cholesterol by ELISA and immunogold electron microscopy.

After development and validation of an ELISA for detecting antibodies to purified cholesterol, naturally occurring antibodies to cholesterol were found in sera from normal humans [4] and pigs, but not guinea pigs [5]. As shown in Fig. 1, using the ELISA technique with crystalline cholesterol as an antigen, we have now assayed sera from 742 preimmunization bleedings obtained from military personnel prior to testing of an unrelated vaccine. Every sample contained easily detectable antibodies to cholesterol, thus extending our previous studies that suggested that naturally occurring IgM and IgG antibodies to cholesterol are present in virtually all normal human sera [4].

The ubiquitous presence of naturally occurring antibodies to cholesterol in sera from young adults suggested the possibility that antibodies to cholesterol might play some role in the metabolic regulation of serum cholesterol, perhaps even a hormone-like role. Several early reports showed that immunization with heterologous β -lipoproteins [6], or a heterologous protein-cholesteryl ester antigen in which cholesteryl sebacate was esterified to a heterologous protein carrier [7,8], inhibited the development of diet-induced hypercholesterolemia and aortic atherosclerosis in rabbit and cockerel models. In the present study, to test whether antibodies produced against liposomal cholesterol might

19950707 048

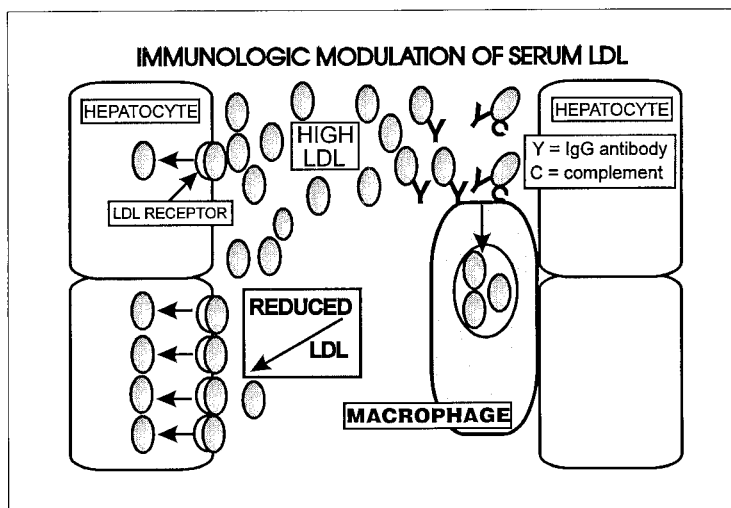


Fig. 4. Proposed mechanism of immunological modulation of diet-induced hypercholesterolemia.

in the serum was lost, presumably due to the binding of the antibodies to the VLDL/IDL that appeared in large amounts in the rabbit sera. Separate experiments demonstrated by ELISA that the antibodies did bind to purified rabbit VLDL/IDL obtained from serum of nonimmunized cholesterol-fed rabbits. Loss of the ability to detect antibodies at 6 weeks by ELISA did not occur with sera from rabbits fed a normal diet not supplemented with cholesterol (Fig. 2, inset). Similar patterns to those observed for IgG antibodies were also observed for IgM antibodies.

Although all the rabbits developed very high cholesterol levels (as high as 3,000 mg/dl in the nonimmunized animals at 12 weeks), the levels in the immunized animals were significantly and substantially lower than the nonimmunized animals (e.g., 1,770 mg/dl for immunized vs. 2,749 mg/dl for nonimmunized at 10 weeks, $p = 0.001$ by *t*-test).

The effect of immunization of rabbits against cholesterol on the subsequent development of aortic fatty streak lesions was determined. 40 animals were immunized monthly (0, 4, 8, and 12 weeks), and 5 weeks after completion of immunization the animals were placed on a 0.5% cholesterol diet for 12 weeks. The results showed markedly less sudanophilia in the immunized animals (Fig. 3). Although approximately 37% less sudanophilia was observed through the entire aortic surface, statistically significant less sudanophilia was demonstrated, 62 and 57% less respectively, in the descending thoracic aorta, and in the abdominal aorta (including the left and right renal arteries), both of which regions are magnified in Fig. 3.

Discussion

The results from the rabbit model demonstrate that immunization with a protein-free liposome formulation containing highly purified nonoxidized cholesterol and lipid A induces IgM and IgG antibodies that recognize both crystalline cholesterol and VLDL/IDL. The immunization procedure also provides prophylactic protection against diet-induced hypercholesterolemia and atherosclerosis. Figure 4 illustrates a mechanism that we believe can explain the experimental observations. We propose that the induced

antibodies can bind to cholesterol present in circulating LDL (or VLDL or IDL), thereby opsonizing the lipoproteins for removal by scavenger macrophages, principally Kupffer cells in the liver. The reduction of serum LDL then results in an upregulation in the number of LDL receptors [11], lowering the LDL still more and causing a further amplification of the beneficial effects.

Although it is true that the rabbit model might be considered somewhat unrealistic when compared with cholesterol levels that might occur in humans, the results suggest that immunization with liposomal cholesterol has tremendous potential potency in that it was able to lower the rabbit serum cholesterol level by as much as 979 mg/dl. This suggests that the immunization procedure might be an effective means of limiting the increases in serum cholesterol induced by diet in humans.

References

1. Sachs H, Klopstock A. *Biochem Z* 1925;159:491-501.
2. Alving CR, Swartz GM Jr. *CRC Crit Rev Immunol* 1991;10:441-453.
3. Swartz GM Jr, Gentry MK, Amende LM, Blanchette-Mackie EJ, Alving CR. *Proc Natl Acad Sci USA* 1988;85:1902-1906.
4. Alving CR, Swartz GM Jr, Wassef NM. *Biochem Soc Trans* 1989;17:637-639.
5. Wassef NM, Johnson SH, Graeber GM, Swartz GM Jr, Schultz CL, Hailey JR, Johnson AJ, Taylor DG, Ridgway RL, Alving CR. *J Immunol* 1989;143:2990-2995.
6. Gero S, Gergely J, Jakab L, Szekely J, Virag S, Farkas K, Czuppon A. *Lancet* 1959;ii:6-7.
7. Bailey JM, Bright R, Tomar R. *Nature* 1964;201:407-408.
8. Bailey JM, Butler J. In: Di Luzio NR, Paoletti R (eds) *The Reticuloendothelial System and Atherosclerosis*. New York: Plenum, 1967;433-441.
9. Cornhill JF, Barrett WA, Herderick EE, Mahley RW, Fry DL. *Atherosclerosis* 1985;5:415-426.
10. Kolodgie FD, Wilson PS, Cornhill JF, Herderick EE, Mergner WJ, Virmani R. *Toxicologic Pathol* 1993; 21:425-435.
11. Brown MS, Goldstein JL. *Proc Natl Acad Sci USA* 1979;76:3330-3337.

Accession For	
NTIS CRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and/or Special
A-1	20